CLAIMS

1. Method of determining the copy number of a nucleotide sequence I in a sample using an amplification technique, said method comprising the steps of

1) adding nucleotides, primers, polymerase and any further reagents, if any, required for the amplification technique used to the sample,

2) performing one or more amplification cycles to amplify the nucleotide sequence I for which the copy number has to be determined;

where the sample contains a chromosomal second nucleotide sequence II, and

a) the first nucleotide sequence I is amplified,

b) the second nucleotide sequence II is amplified,

c) a third nucleotide sequence I' corresponding to the first nucleotide sequence I and present in a control sample is amplified at various dilutions, and

d) a fourth nucleotide sequence II' corresponding to the second nucleotide sequence II and present in a control sample is amplified at various dilutions,

where the ratio of the concentrations of nucleotide sequence I' and II' is known

where the amplifications of the third and fourth nucleotide sequences I' and II' at various dilutions allows standard curves SC_i with i being I or II to be made, the concentrations of I and II are determined by using the respective standard curve SC_i , and the relative concentrations allows the relative copy number CN of sequence I (versus nucleotide sequence II) to be determined using the formula

$$CN = \frac{[I]_{SC_{I'}}}{[II]_{SC_{II'}}}$$

where

CN is the relative copy number of I over II in the sample;

$[I]_{SC_I}$, is the concentration of I determined using standard curve SC_I; and

$[II]_{SC_{II}}$, is the concentration of II determined using standard curve SC_{II},

wherein

at least one pair of amplification reactions chosen from i) a) and b), and ii) c) and d) is performed in a single container and monitored spectrophotometrically during amplification, and

the third nucleotide sequence I' and fourth nucleotide sequence II' resides on a single vector.

2. Method according to claim 1, **characterized** in that the absolute copy number is determined by multiplying the copy number CN by the absolute copy number of sequence II per cell.

3. Method according to claim 1 or 2, **characterized** in that at least two and also more different third nucleotide sequences I' for measuring a corresponding number of different first nucleotide sequences I reside on a single vector.

4. Method according to any of the preceding claims, **characterized** in that the sequence of the first nucleotide sequence I is the same as the third nucleotide sequence I'.

5. Method according to any of the preceding claims, **characterized** in that the sequence of the second nucleotide sequence II is the same as the fourth nucleotide sequence II'.